BJR

Received: 13 February 2017 | Revised: 28 March 2017 | Accepted: 30 March 2017

https://doi.org/10.1259/bjr.20170113

Cite this article as:
Hutchinson JC, Shelmerdine SC, Simcock IC, Sebire NJ, Arthurs OJ. Early clinical applications for imaging at microscopic detail: microfocus computed tomography (micro-CT). *Br J Radiol* 2017; **90**: 20170113.

REVIEW ARTICLE

Early clinical applications for imaging at microscopic detail: microfocus computed tomography (micro-CT)

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ABSTRACT

Microfocus CT (micro-CT) has traditionally been used in industry and preclinical studies, although it may find new applicability in the routine clinical setting. It can provide high-resolution three-dimensional digital imaging data sets to the same level of detail as microscopic examination without the need for tissue dissection. Micro-CT is already enabling non-invasive detailed internal assessment of various tissue specimens, particularly in breast imaging and early gestational fetal autopsy, not previously possible from more conventional modalities such as MRI or CT. In this review, we discuss the technical aspects behind micro-CT image acquisition, how early work with small animal studies have informed our knowledge of human disease and the imaging performed so far on human tissue specimens. We conclude with potential future clinical applications of this novel and emerging technique.

INTRODUCTION

Since the inception of CT in the 1970s, the diagnosis and treatment pathways of many medical conditions have been revolutionized.[1] Whilst current state-of-the art CT scanners can image structures down to a voxel size of 1 mm^3, the need for higher resolution imaging for smaller specimens led to the first microfocus CT (micro-CT) scanners being developed in the 1980s.[2]

Over the subsequent decades, micro-CT imaging technology has advanced considerably with current scanners able to achieve sub-micron voxel-level resolution. The increasing availability and usage of this technique in modern science is reflected by the rising number of publications on the topic.[3] This has been predominantly due to work in small animal studies which include phenotyping,[4–6] bone morphology analysis[7] and plant biology.[8]

Micro-CT has also been used extensively in industry for non-destructive precision engineering, particularly where strict tolerances are required, or where performance of a part is vital to the safe function of a system (*e.g.* aircraft engine turbine blade inspection).[9] It also plays an important role in archaeology, where historical artefacts can be analyzed and "virtually dissected" to investigate their contents, thereby minimizing disruption and degradation due to handling.[10]

Owing to the requirement for relatively long scan times (which can range between 8 min and 3 h depending on resolution and image quality) and need to mechanically stabilize the object being scanned, micro-CT is not yet widely utilized in clinical investigations. Nevertheless, this may change as technology advances, knowledge of the technique develops and costs of scanner manufacture and maintenance reduce.

The objectives of this review article are to introduce readers to the principles of this novel imaging technique, how it has been utilized for preclinical usage to date and where this modality may benefit medical practice in the future.

Technical aspects

Micro-CT scanners work in a similar manner to medical CT scanners, in that both methods rely on an X-ray beam to irradiate the object of interest and photosensitive detectors record the unabsorbed photon signals to produce an interpretable three-dimensional (3D) data set after software post-processing techniques are applied (*e.g.* modified filtered back projection).

Two main construction designs principles are in existence (Figure 1). The first (sometimes termed "minifocus CT") is an identical setup to medical CT scanners with the X-ray detection source and detectors mounted on a rotating gantry

around the object being examined. In these systems, there is normally a fixed "radiation source-to-detector" distance which limits the resolution capabilities (approximate range of 50–100 micron voxels). In the second design (used for industrial applications and most *ex vivo* specimens) (Figure 2) the radiation source is fixed, with the object of interest mounted on an adjustable, rotating platform during the examination. The advantage of this is that this allows for the adjustment of the "radiation source-to-object" and "object-to-detector" distance, giving improved resolution (less than 1 micron voxels achievable). Resolution comparable with high-power histology can be achieved with adequate magnification. There is also the additional benefit of an isotropic 3D data set that does not require dissection of the specimen or labour-intensive practices, which would be necessary for high-resolution episcopic microscopy and episcopic fluorescence image capture.

Microfocus CT contrast media

As soft tissue demonstrates minimal inherent X-ray attenuation, many micro-CT studies utilize either immersion of the tissues in exogenous contrast or long exposure times and frame averaging in order to differentiate between tissues and tissue substructures.

Proposed tissue contrast agents for use with micro-CT include iodine preparations [including Lugol's solution/I_2KI, elemental iodine dissolved in ethanol or methanol (I_2E/I_2M) and potassium iodide], phosphotungstic acid, phosphomolybdic acid, osmium tetroxide and other metal-based compounds (*e.g.* mercuric chloride, silver nitrate).[4,5,11] Contrast agents vary widely in their tissue specificity, cost, toxicity and effect on the subsequent production of histology preparations; therefore, the choice of tissue contrast is a key consideration.

Other silicone based compounds that solidify following injection into the vascular system and addition of a curing agent may be used for angiographic investigations. When appropriately used, a contrast agent may allow for shorter scans with better identification of differential densities from target tissues. Non-contrast (unenhanced) scans can also provide excellent results, even in low-density tissues such a lung and skin.[12–14] As paraffin-embedded tissue samples are mechanically stable and not amenable to immersion in an exogenous agent, they are also well suited to non-contrast techniques.

Where larger structures (such as human fetuses) are examined, the variety of different tissue types within the structure greatly benefit from contrast emersion and provide excellent differentiation of internal viscera currently below the level offered by post-mortem CT and MRI. Initial proof of principle has been demonstrated,[15,16] although technical considerations and diagnostic accuracy remain to be addressed.

EVALUATION OF MICROFOCUS CT FOR ANIMAL TISSUE

Advances in genetic engineering have resulted in multiple animal models of human diseases, particularly in drug development, treatment and the pre-clinical understanding of disease manifestations. Recent optimization of contrast agent development and administration now permit vessel morphology and soft tissue structures *in vivo* to be evaluated.[17] Nevertheless, *ex vivo* animal imaging still confers certain advantages over *in vivo* imaging, particularly increased resolution, partly from longer scan times (which can take up to 2–3 h for a highly detailed study) and increased photon flux without the

Figure 1. Microfocus (micro-CT) machine construction principles: (a) a "mini-focus" CT design and a (b) typical micro-CT used for industrial imaging and preclinical research are demonstrated. The mini-focus CT design is similar to that of a medical CT scanner, whereas in the second design the object is being rotated and different parameters such as "source-to-object" and "object-to-detector" distances are adjustable allowing for greater magnification and resolution of the resultant image.

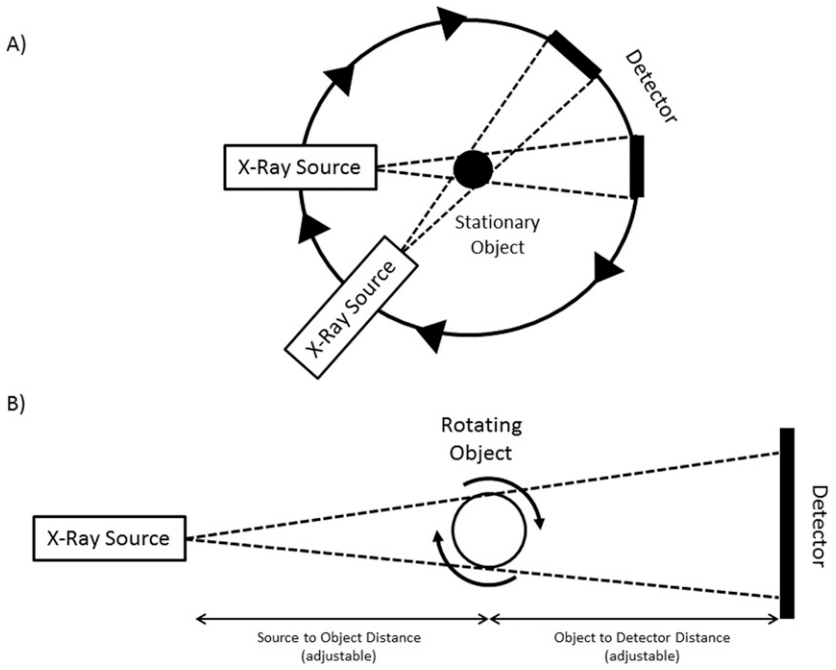
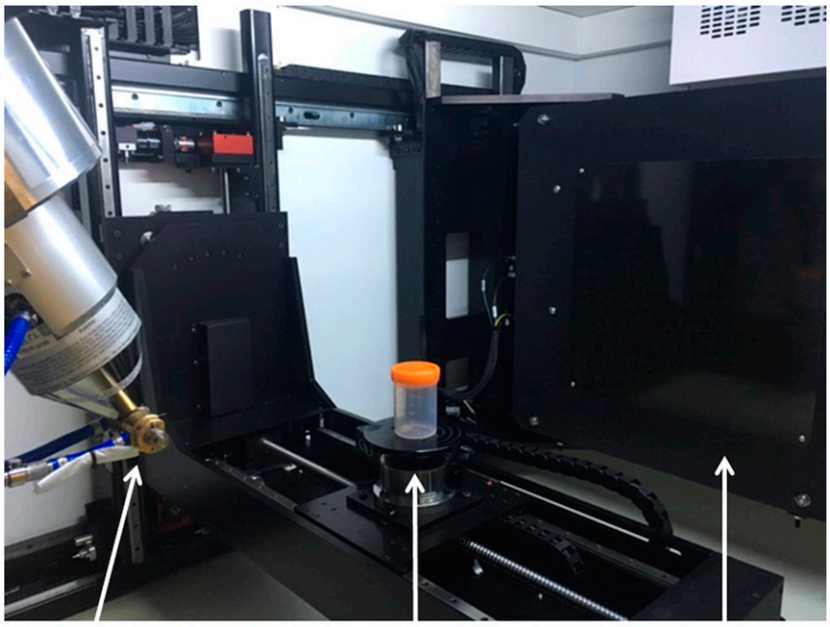


Figure 2. Inside a microfocus CT machine: the radiation source (X-ray gun), rotating platform (with specimen pot mounted) and detector are visible to the operator and labelled accordingly. All of these components are housed within a radiation-shielding cabinet made of lead and steel, which means there is no need for additional lead shielding when using the machinery, nor for it to be placed in a specialized lead-lined room.



consideration of anaesthetic requirements, cardiac gating or increased radiation dosage.[18] Here, we review the use of micro-CT imaging in animal models and highlight how information acquired may be translated into the understanding and treatment of human diseases.

Neurological

Several *ex vivo* studies have provided detailed cerebral vascular anatomy,[19] estimation of cerebral blood volume and detection of atherosclerotic lesions in the mouse brain.[20] More recently, *in vivo* techniques have allowed imaging of blood–brain barrier dysfunction in mice post-stroke[21] and micropositron emission tomography/CT imaging has proved to be a feasible technique in longitudinal assessment of brain tumour growth and treatment response in mice.[22] Industrial micro-CT machines have also been modified to produce targeted high radiation doses for radiotherapy in mouse models with glioblastoma implants.[23] These models to assess and quantify diseases and improvements with novel therapies have the potential for translation into human trials.

Chest and cardiac

Ex vivo rat and rabbit heart imaging with contrast-enhanced micro-CT have characterized the 3D cardiac conduction system which has the potential to direct surgical treatment planning for congenital heart disease and cardiac ablation procedures.[24] Micro-CT imaging and models could be useful in educating surgeons and cardiologists in interventional cardiac techniques when training on animal models, prior to independently intervening on live patients.

Diagnostic accuracy of micro-CT for the detection of congenital heart disease in mice is almost 90% when compared with histology[25] and micro-CT of human *ex vivo* hearts would improve our understanding of cardiac structure and anatomy, particularly in the less invasive perinatal autopsy setting.

Automated micro-CT software has been used to quantify aerated lung volumes in an *in vivo* mouse model of bleomycin-induced pulmonary fibrosis with good repeatability and potential for drug studies,[26] and also to demonstrate tumour progression and response to experimental therapy in a mouse model of lung cancer.[27] These assessments of treatment response may again help to develop novel therapies translatable to humans.

Abdominal

Models mimicking colonic carcinoma in mice have shown a high correlation between identified tumour volume and histology[28] on micro-CT colonography. If this is translatable in human diseases, the imaging and assessment of surgical specimens with micro-CT alone may remove the need for histological dissection of the specimen.

The use of liver-specific iodinated contrast agents *in vivo* in mice models has also allowed for delineation between healthy "enhancing" hepatocytes and unenhancing neoplastic lesions (as small as 100 μm), thereby allowing quantification of liver metastases,[29,30] and changes in size and number with experimental drug trials. Detailed 3D illustrations of the murine liver for research anatomical reference[31] has also been described to assist animal researchers in determining the vascular supply to

the liver and potential hepatic lesions. This enables accurate assessment of tumour spread and relapse in models of disease and thereby identification of treatment failure.

Musculoskeletal and soft tissue

Numerous articles have investigated the effects of bone structure, osteogenesis, osteoporosis, bone resorption, bone remodelling, fracture healing, neoplasms and biocompatible materials,[3,7,32,33] within various animal models. The ability of micro-CT to evaluate trabecular structures allows quantification of bone destruction, commonly assessed in drug treatments and oncological progression.[34] Mouse models with osteogenesis imperfecta have demonstrated improved bone architecture with haematopoietic stem cell transplant therapy[35] and characterization of osseous tumour infiltration have been combined with bioluminescence imaging techniques in prostate cancer mouse models *in vivo* to better assess tumour growth and response.[19,36] There are few studies of micro-CT assessment of muscle fibre characterization and disease, although its feasibility and benefits over diffusion tensor imaging have been reported.[37]

Micro-CT has been used to test the effectiveness of uptake of implant materials within muscle tissue "dead space" post-debridement in mice.[38] This information can be used to inform the degree of soft tissue compatibility with various prosthetic and surgical materials. Other animal studies using micro-CT to characterize changes in muscle angiogenesis and fatty muscle infiltration post-distraction osteogenesis[39] and muscle tenotomy,[40] respectively, could interest orthopaedic surgeons and rheumatologists on potential post-operative processes occurring in their patients.

Genetic diseases

Early research has shown that micro-CT imaging of embryonic mice analyzed with autorecognition software can help segment various organs to build a database of standard organ dimensions for embryonic development. As there is 99% homology between mice and humans, researchers hope that different mutated mouse lines can be imaged and organ volumes evaluated to enable better understanding of the contributory role each gene plays in different organ development. This may have implications for prognosis when identifying human genetic disorders *in utero*.[41]

EVALUATION OF MICROFOCUS CT FOR HUMAN TISSUE

Phenotyping of human disease using micro-CT is less well established than in animal models, although these techniques are gaining popularity and could offer new insights into human pathology.

Neurological

One *ex vivo* study has demonstrated the ability of micro-CT to identify various cellular constituents of adamantinomatous craniopharyngioma tumours within brain tissue, based on grey levels (Figure 3). Post-processing volumes allowed the complex 3D relationships of the tumour to be demonstrated within the background brain tissue at isotropic voxel sizes of 4–6 microns.[42] Subsequent histological processing and staining was not affected by iodine-enhanced micro-CT examination, which is encouraging for wider application of histological validation of micro-CT findings.

Chest and cardiac

Two studies examining the use of micro-CT for human fetal *ex vivo* hearts show that iodine-enhanced micro-CT can provide highly accurate 3D renderings of a variety of complex congenital heart diseases, without the need for injection of contrast agents and detailed tissue dissection. Initial diagnostic accuracy data suggest that in isolated human fetal hearts (or heart and lung blocks) extracted at autopsy, micro-CT has >95% concordance with full autopsy for anatomical features, with no apparent discrepancies in overall diagnosis between micro-CT and full autopsy in these feasibility studies (Figure 4).[15,43–45]

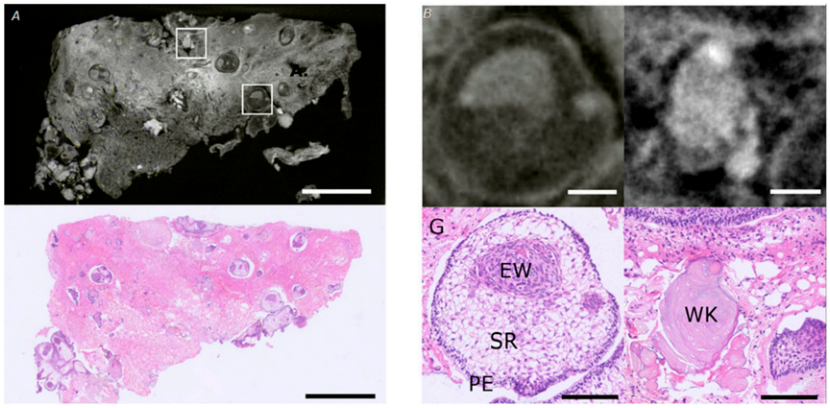
The potential for 3D data sets to enhance traditional histopathology methods is also demonstrated in studies that used non-contrast micro-CT to examine lung tissues. Scott et al[12] demonstrate 3D modelling of small airways and blood vessels within formalin-fixed paraffin-embedded lung tissue that could potentially be interrogated by a morphometric approach for investigation of disease processes (Figure 5).

Further to this work, features of lung pathology, such as fibroblastic foci, which are important diagnostic features of some interstitial lung diseases, have been identified on micro-CT examination of formalin-fixed paraffin-embedded lung tissue and their 3D relationships interrogated. Jones et al[14] demonstrated no evidence of interconnectivity between fibroblastic foci in samples taken from patients with idiopathic pulmonary fibrosis, consistent with the current concept that fibroblastic foci represent discrete sites of repair following lung injury. Applying a similar morphometric approach to explanted frozen lung tissue, loss of pre-terminal and terminal bronchioles in patients with lung allograft dysfunction has also been identified using micro-CT and verified using comparative histology.[46] Further analysis of frozen lungs explanted from patients with cystic fibrosis has yielded detailed information of end-stage cystic fibrosis disease showing dilatation and obstruction of nearly all airway generations down to the terminal bronchioles.[47]

Abdominal

There are little data regarding micro-CT imaging of human intra-abdominal viscera, with the majority of studies available relating to the kidney. One case report[48] has demonstrated normal micro-CT renal anatomy at autopsy in a term neonate, with resolution comparable with that of low-power histology. In a case of known cystic kidney disease, radially arranged, elongated cysts could be readily defined from the micro-CT data, allowing the diagnosis for autosomal recessive polycystic kidney disease to be made. Imaging of a multicystic dysplastic kidney revealed cysts of varying sizes with histological confirmation. Another study demonstrated imaging of lower urinary tract obstruction using contrast-enhanced micro-CT.[49] Cystic kidney disorders have variable inheritance patterns, with a wide variation in recurrence risk. With further optimization, micro-CT may be able to provide a non-invasive, permanent record of disease phenotype in such cases without the need for a full autopsy.

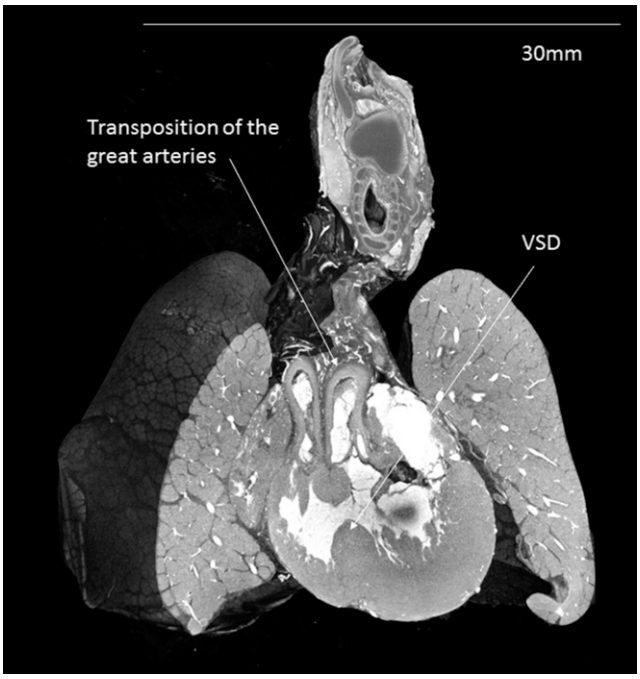
Figure 3. Microfocus CT imaging of human adamantinomatous craniopharyngioma (ACP): (a) virtual and matched histological tissue section of an ACP showing areas of tumour interspersed by reactive glial tissue. Scale bar indicates 1 mm. (b) 20× images of specific tumour compartments from boxed regions of (a). The left panel shows epithelial whorls ("clusters") within an area of tumour and the right panel shows "wet keratin" (WK) which has a higher grey value on CT imaging. Scale bars indicate 100 μm. EW, epithelial whorls; G, reactive glial tissue; PE, palisading epithelium; SR, stellate reticulum. Figure adapted from Apps et al[42] under the Creative Commons Attribution License 4.0.



Musculoskeletal and soft tissue

The majority of published micro-CT work in humans relating to the bone has thus far focused on maxillofacial and dental surgery-related topics. These have included assessments of dental and mandibular morphology,[50] and bone quality at dental implant sites.[51] Outside of dentistry, human cadaveric cochleae have been examined with both micro-CT and synchrotron imaging to create 3D anatomical models which may inform cochlear implant surgery in the future.[52] Another recent study assessed bone cores from 68 paediatric patients with end-stage renal failure using micro-CT. The imaging findings allowed the researchers to accurately measure bone volume and quantify the inverse relationship between osteoid accumulation at bone histomorphometry and bone mineral density from micro-CT measurements.[53]

Figure 4. Microfocus CT volume rendering of a heart and lung block from an 18-gestational week fetus with hypoplastic right heart syndrome: transposition of the great arteries and a ventriculoseptal defect (VSD) can be visualized. Adapted from Hutchinson et al[45] with permission.



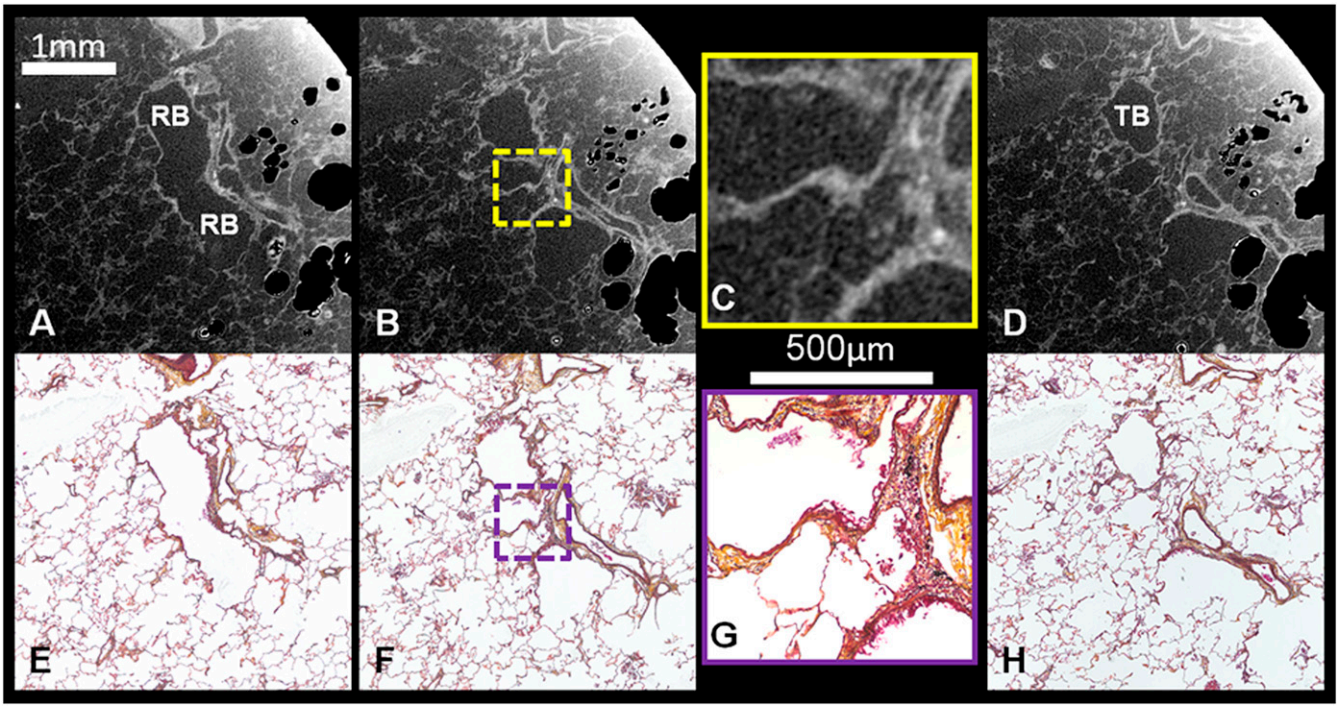
With regard to soft tissue findings, the most exciting work in humans has related to breast imaging studies. Tang et al[54–56] have used micro-CT to assess intraoperative breast lumpectomy specimens to give their surgical colleagues real-time analysis and information on tumour margins. More recent work has shown that breast cancer specimen T-stage assignment by micro-CT analysis only differed in 15.2% of cases when compared with histological analysis; in addition, there was a strong agreement between tumour size and stage for invasive ductal carcinoma between the two techniques, although not for invasive lobular carcinoma.[57]

Human autopsy

The combination of improved ultrasound screening and non-invasive prenatal testing means that decisions regarding the management of pregnancies are more commonly made at the end of the first trimester, when fetuses are usually too small for diagnostic examination using 1.5-T MRI.[58]

Lombardi et al[15] have demonstrated proof of principle of the use of iodine-enhanced micro-CT in human fetal autopsy between the ages of 7–17 gestational weeks (Figure 6). The opportunity to use micro-CT for whole-body early-gestation fetal autopsy represents a novel imaging tool in these cases, to confirm or refute diagnoses leading to termination of pregnancy.[16] In addition, these findings demonstrate the potential of micro-CT for detailed PM imaging of entire fetuses whilst maintaining tissue integrity, with significant implications for perinatal autopsy practice. 3D imaging data sets generated by micro-CT provide a permanent record of findings that can be virtually dissected and discussed with the clinical team.

Figure 5. Matched serial microfocus CT (micro-CT) and Movat's pentachrome-stained histological sections: serial micro-CT images (a–d) and corresponding Movat's pentachrome-stained histological sections (e–h) demonstrate the ability of micro-CT to provide sufficient contrast between tissue and paraffin to allow image analysis. RB, respiratory bronchiole; TB, terminal bronchiole. Adapted from Scott et al[12] in accordance with the Creative Commons Attribution License 4.0.



Forensic applications

Micro-CT also has an emerging role in the forensic investigation of deaths, where utility has been demonstrated across a wide range of applications, including non-destructive phenotyping of bony injuries[59,60] to match wounds with potential instruments. Use of micro-CT to assist entomological investigations could potentially assist in estimating a minimum post-mortem death interval through the features of blowfly larvae present on a body.[61] Features of tooth development could also potentially be assessed to assist with age estimation. Cecchetto et al[62,63] have also demonstrated the use of micro-CT to examine gunshot residue and wounds in various forensic scenarios.[64]

In one interesting case, Fais et al[65] describe a situation where micro-CT demonstration of subtle laryngeal fractures and intracartilaginous laryngeal haemorrhages (not seen on conventional multislice CT imaging or evidenced by visual external examination) allowed confirmation of fatal manual strangulation as mode of death where the perpetrators confession was deemed unreliable in court owing to mental illness. However, this is an exceptional situation, and the use of novel imaging techniques in court should be approached with caution prior to rigorous and sound scientific merit on accuracy of the procedure.

LIMITATIONS OF MICRO-CT

Several issues remain to be addressed for widespread implementation of micro-CT scanning of complete early human fetuses. These include, but are not limited to, the cost of the procedure and equipment (including processes for reimbursement), the effects of tissue colouration and distortion due to the fixation and contrasting processes, the current need for a skilled operator to acquire, reconstruct and process the images and data storage requirements in orders of magnitude greater than conventional clinical scans (this typically ranges from 10 to 30 GB per case).

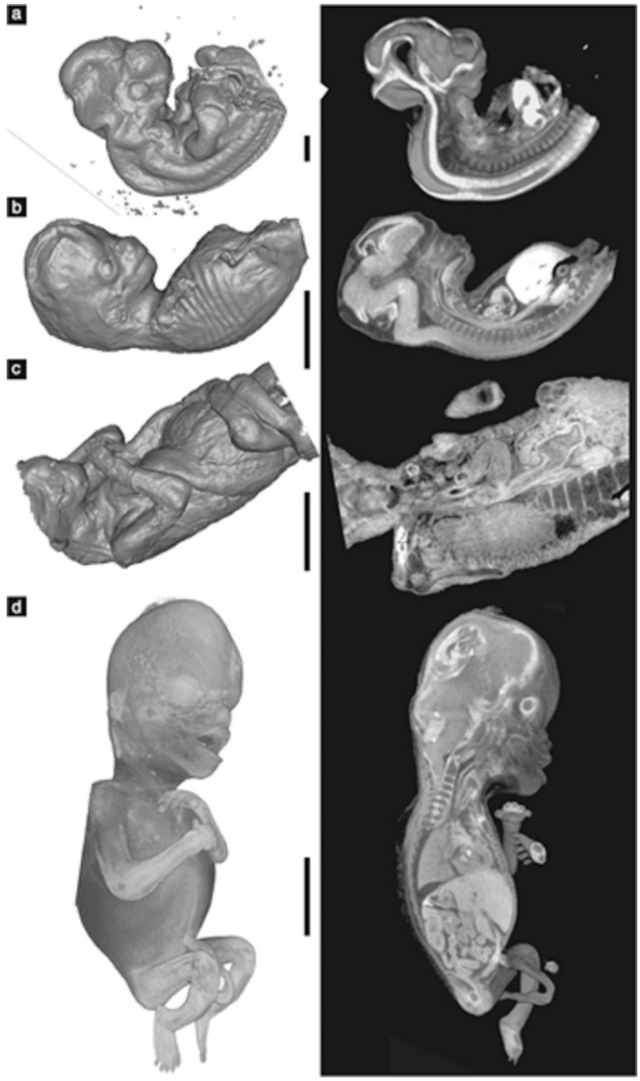
Some of the reported human fetal studies have used eviscerated organs in cases undergoing full standard autopsy as proof of principle, whereas a true non-invasive or less-invasive autopsy would involve whole-body *in situ* organ imaging. Further studies will be needed to optimize tissue preparation as well as image acquisition in a variety of clinical settings, prior to comprehensive formal diagnostic accuracy evaluations against both existing imaging and pathology techniques.

FUTURE DIRECTIONS

Once the use of micro-CT becomes cheaper and more widespread, the potential for clinical applications are far reaching and promising for educational, diagnostic and therapeutic monitoring purposes.

In research, preparing and analyzing micro-CT images alongside traditional histopathological laboratory techniques has shown excellent agreement. This allows for a standardized and reproducible methodology, with the added benefit of revisiting the digital imaging data by other researchers, remotely if desired. It could improve consistency between multicentre study results, as prior studies of histological specimens have reported different preparation techniques between laboratories limiting the generalizability of findings.

Figure 6. Contrast-enhanced microfocus CT of fetuses from 7 to 15 weeks of gestation, at resolution of 18 μm: three-dimensional surface rendering is shown on the left, and the internal anatomical details obtained with volume sections are shown on the right. (a) Fetus 1, 7 weeks; (b) Fetus 3, 10 weeks; (c) Fetus 4, 11 weeks; and (d) Fetus 6, 15 weeks. Scale bar in (a) is 1 mm and in (b–d), it is 10 mm. Reproduced from Lombardi et al[15] with permission.



Building on this knowledge, micro-CT analysis can be implemented in routine clinical work where imaging of intraoperative surgical specimens (such as in the study described for breast specimens) may inform surgical resection margins in real time. This intraoperative approach can be postulated to be useful in other contexts, such as children undergoing nephron-sparing surgery for Wilms tumour resection. Accurate differentiation of tumour constituents within an excised tumour specimen may be possible by adapting the techniques described in human pituitary tumour specimens, facilitating more detailed or specific tumour characterization. Analysis of tumour angiogenesis and invasion can also be reviewed in surgical specimens following resection without the need for complex staining techniques to provide information of spread and treatment effects (for example in brain tumours). Micro-CT imaging offers the added advantage over specimen dissection of virtual slicing of the tissue in any plane and providing 3D relationships of different tissue compartments.

Future optimization and development of *in vivo* micro-CT machines may make it feasible to replace invasive methods of assessing bone microarchitecture, such as bone histomorphometry, which relies on obtaining a bone biopsy. Micro-CT may help differentiate different bone pathologies based on trabeculation and bone mineralization densities and could monitor the effects of treatment from metabolic bone diseases (such as changes with bisphosphonate therapy). Nevertheless, this would still require more refinement of the technique, and studies are yet to show that micro-CT can differentiate pathology from normal bone samples in humans.

CONCLUSION

Micro-CT is an established animal and industrial imaging technique which is now being evaluated in the human setting, with early reports of diagnostic evaluation. Micro-CT has the potential to provide a step-change improvement in diagnostic imaging of small clinical specimens, with near histological levels of detail. As a non-destructive imaging modality, videos and 3D images can be easily stored, re-examined and transferred to specialists for diagnostic opinions, unlike current histological evaluations. The combination of high anatomical detail, relatively short scan times and computer-aided dissection could put micro-CT at the centre of human fetal autopsy examinations and surgical specimen imaging in the future.

FUNDING

OJA is supported by a NIHR Clinician Scientist Fellowship awards (NIHR-CS-012-002), and receives funding from the Great Ormond Street Hospital Children's Charity and NIHR GOSH Biomedical Research Centre. This article presents independent research funded by the National Institute for Health Research (NIHR) and supported by the Great Ormond Street Hospital Biomedical Research Centre.

REFERENCES

1. Ritman EL. Micro-computed tomography—current status and developments. *Ann Rev Biomed Eng* 2004; **6**: 185–208. doi: https://doi.org/10.1146/annurev.bioeng.6.040803.140130

2. Cavanaugh D, Johnson E, Price RE, Kurie J, Travis EL, Cody DD. *In vivo* respiratory-gated micro-CT imaging in small-animal oncology models. *Mol Imaging* 2004; **3**: 55–62. doi: https://doi.org/10.1162/153535004773861723

3. Shambach SJ, Bag S, Schilling L, Groden C, Brockmann MA. Application of micro-CT in small animal imaging. *Methods* 2010; **50**: 2–13. doi: https://doi.org/10.1016/j.ymeth.2009.08.007

4. Metscher BD. MicroCT for developmental biology: a versatile tool for high-contrast 3D imaging at histological resolutions. *Dev Dyn* 2009; **238**: 632–40.
5. Metscher BD. MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. *BMC Physiol* 2009; **9**: 11.
6. Degenhardt K, Wright AC, Horng D, Padmanabhan A, Epstein JA. Rapid 3D phenotyping of cardiovascular development in mouse embryos by micro-CT with iodine staining. *Circ Cardiovasc Imaging* 2010; **3**: 314–22. doi: https://doi.org/10.1161/circimaging.109.918482
7. Ito M. Assessment of bone quality using micro-computed tomography (micro-CT) and synchrotron micro-CT. *J Bone Miner Metab* 2005; **23**: 115–21.
8. Mairhofer S, Zappala S, Tracy S, Sturrock C, Bennett MJ, Mooney SJ, et al. Recovering complete plant root system architectures from soil *via* X-ray mu-computed tomography. *Plant Methods* 2013; **9**: 8. doi: https://doi.org/10.1186/1746-4811-9-8
9. Bergmann RB, Bessler FT, Bauer W. Non-destructive testing in the automotive supply industry—requirements, trends and examples using X-ray CT. In: 9th European Conference on Non-Destructive Testing: Berlin, Germany; 2006.
10. Freeth T, Bitsakis Y, Moussas X, Seiradakis JH, Tselikas A, Mangou H, et al. Decoding the ancient Greek astronomical calculator known as the antikythera mechanism. *Nature* 2006; **444**: 587–91.
11. Gignac PM, Kley NJ, Clarke JA, Colbert MW, Morhardt AC, Cerio D, et al. Diffusible iodine-based contrast-enhanced computed tomography (diceCT): an emerging tool for rapid, high-resolution, 3-D imaging of metazoan soft tissues. *J Anat* 2016; **228**: 889–909. doi: https://doi.org/10.1111/joa.12449
12. Scott AE, Vasilescu DM, Seal KA, Keyes SD, Mavrogordato MN, Hogg JC, et al. Three dimensional imaging of paraffin embedded human lung tissue samples by micro-computed tomography. *PLoS One* 2015; **10**: e0126230.
13. Walton LA, Bradley RS, Withers PJ, Newton VL, Watson RE, Austin C, et al. Morphological characterisation of unstained and intact tissue micro-architecture by X-ray computed micro- and nano-tomography. *Sci Rep* 2015; **5**: 10074.
14. Jones MG, Fabre A, Schneider P, Cinetto F, Sgalla G, Mavrogordato M, et al. Three-dimensional characterization of fibroblast foci in idiopathic pulmonary fibrosis. *JCI Insight* 2016; **5**: e86375.
15. Lombardi CM, Zambelli V, Botta G, Moltrasio F, Cattoretti G, Lucchini V, et al. Postmortem microcomputed tomography (micro-CT) of small fetuses and hearts. *Ultrasound Obstet Gynecol* 2014; **44**: 600–9. doi: https://doi.org/10.1002/uog.13330
16. Hutchinson J, Arthurs OJ, Haig IG, Sebire NJ. Novel clinical autopsy performed with post-mortem micro-CT: first experience. *J Pathol* 2016; **238**: 11.
17. Stiller W, Kobayashi M, Koike K, Stampfl U, Richter GM, Semmler W, et al. Initial experience with a novel low-dose micro-CT system. *Rofo* 2007; **179**: 669–75. doi: https://doi.org/10.1055/s-2007-963189
18. Dorr A, Sled JG, Kabani N. Three-dimensional cerebral vasculature of the CBA mouse brain: a magnetic resonance imaging and micro computed tomography study. *Neuroimage* 2007; **35**: 1409–23. doi: https://doi.org/10.1016/j.neuroimage.2006.12.040
19. Fritz V, Louis-Plence P, Apparailly F, Noel D, Voide R, Pillon A, et al. Micro-CT combined with bioluminescence imaging: a dynamic approach to detect early tumor-bone interaction in a tumor osteolysis murine model. *Bone* 2007; **40**: 1032–40. doi: https://doi.org/10.1016/j.bone.2006.11.026
20. Dorr AE, Lerch JP, Spring S, Kabani N, Henkelman RM. High resolution three-dimensional brain atlas using an average magnetic resonance image of 40 adult C57Bl/6J mice. *Neuroimage* 2008; **42**: 60–9. doi: https://doi.org/10.1016/j.neuroimage.2008.03.037
21. Langheinrich AC, Michniewicz A, Bohle RM, Ritman EL. Vasa vasorum neovascularization and lesion distribution among different vascular beds in ApoE−/−/LDL−/− double knockout mice. *Atherosclerosis* 2007; **191**: 73–81. doi: https://doi.org/10.1016/j.atherosclerosis.2006.05.021
22. Park JY, Lee SK, Kim JY, Je KH, Schellingerhout D, Kim DE. A new micro-computed tomography-based high-resolution blood-brain barrier imaging technique to study ischemic stroke. *Stroke* 2014; **45**: 2480–4. doi: https://doi.org/10.1161/strokeaha.114.006297
23. Park SS, Chunta JL, Robertson JM, Martinez AA, Oliver Wong CY, Amin M, et al. MicroPET/CT imaging of an orthotopic model of human glioblastoma multiforme and evaluation of pulsed low-dose irradiation. *Int J Radiat Oncol Biol Phys* 2011; **80**: 885–92. doi: https://doi.org/10.1016/j.ijrobp.2011.01.045
24. Felix MC, Fleckenstein J, Kirschner S, Hartmann L, Wenz F, Brockmann MA, et al. Image-guided radiotherapy using a modified industrial micro-CT for preclinical applications. *PLoS One* 2015; **10**: e0126246. doi: https://doi.org/10.1371/journal.pone.0126246
25. Stephenson RS, Boyett MR, Hart G, Nikolaidou T, Cai X, Corno AF, et al. Contrast enhanced micro-computed tomography resolves the 3-dimensional morphology of the cardiac conduction system in mammalian hearts. *PLoS One* 2012; **7**: e35299. doi: https://doi.org/10.1371/journal.pone.0035299
26. Kim A, Francis R, Liu X, Devine W, Ramirez R, Anderton S, et al. Microcomputed tomography provides high accuracy congenital heart disease diagnosis in neonatal and fetal mice. *Circ Cardiovasc Imaging* 2013; **6**: 551–9. doi: https://doi.org/10.1161/circimaging.113.000279
27. De Langhe E, Vande Velde G, Hostens J, Himmelreich U, Nemery B, Luyten FP, et al. Quantification of lung fibrosis and emphysema in mice using automated micro-computed tomography. *PLoS One* 2012; **7**: e43123. doi: https://doi.org/10.1371/journal.pone.0043123
28. Haines BB, Bettano KA, Chenard M, Sevilla RS, Ware C, Angagaw MH, et al. A quantitative volumetric micro-computed tomography method to analyze lung tumors in genetically engineered mouse models. *Neoplasia* 2009; **11**: 39–47. doi: https://doi.org/10.1593/neo.81030
29. Durkee BY, Mudd SR, Roen CN, Clipson L, Newton MA, Weichert JP, et al. Reproducibility of tumor volume measurement at microCT colonography in living mice. *Acad Radiol* 2008; **15**: 334–41. doi: https://doi.org/10.1016/j.acra.2007.10.005
30. Almajdub M, Nejjari M, Poncet G, Magnier L, Chereul E, Roche C, et al. *In-vivo* high-resolution X-ray microtomography for liver and spleen tumor assessment in mice. *Contrast Media Mol Imaging* 2007; **2**: 88–93. doi: https://doi.org/10.1002/cmmi.130
31. Montet X, Pastor CM, Vallee JP, Becker CD, Geissbuhler A, Morel DR, et al. Improved visualization of vessels and hepatic tumors by micro-computed tomography (CT) using iodinated liposomes. *Invest Radiol* 2007; **42**: 652–8. doi: https://doi.org/10.1097/rli.0b013e31805f445b
32. Morgan EF, Mason ZD, Chien KB, Pfeiffer AJ, Barnes GL, Einhorn TA, et al. Micro-computed tomography assessment of fracture healing: relationships among callus structure, composition, and mechanical function. *Bone* 2009; **44**: 335–44.

doi: https://doi.org/10.1016/j.bone.2008.10.039
33. Wise LD, Winkelmann CT, Dogdas B, Bagchi A. Micro-computed tomography imaging and analysis in developmental biology and toxicology. *Birth Defects Res C Embryo Today* 2013; **99**: 71–82. doi: https://doi.org/10.1002/bdrc.21033
34. Geffre CP, Pond E, Pond GD, Sroka IC, Gard JM, Skovan BA, et al. Combined micro CT and histopathology for evaluation of skeletal metastasis in live animals. *Am J Transl Res* 2015; **7**: 348–55.
35. Mehrotra M, Rosol M, Ogawa M, Larue AC. Amelioration of a mouse model of osteogenesis imperfecta with hematopoietic stem cell transplantation: microcomputed tomography studies. *Exp Hematol* 2010; **38**: 593–602. doi: https://doi.org/10.1016/j.exphem.2010.04.008
36. Tiwari S, Schem C, Lorenzen AC, Kayser O, Wiese C, Graeff C, et al. Application of *ex vivo* micro-computed tomography for assessment of *in vivo* fluorescence and plain radiographic imaging for monitoring bone metastases and osteolytic lesions. *J Bone Miner Metab* 2012; **30**: 373–80. doi: https://doi.org/10.1007/s00774-011-0335-z
37. Jeffery NS, Stephenson RS, Gallagher JA, Jarvis JC, Cox PG. Micro-computed tomography with iodine staining resolves the arrangement of muscle fibres. *J Biomech* 2011; **44**: 189–92. doi: https://doi.org/10.1016/j.jbiomech.2010.08.027
38. Oliver RA, Lovric V, Yu Y, Christou C, Aiken SS, Cooper JJ, et al. Development of a novel model for the assessment of dead-space management in soft tissue. *PLoS One* 2015; **10**: e0136514. doi: https://doi.org/10.1371/journal.pone.0136514
39. Morgan EF, Hussein AI, Al-Awadhi BA, Hogan DE, Matsubara H, Al-Alq Z, et al. Vascular development during distraction osteogenesis proceeds by sequential intramuscular arteriogenesis followed by intraosteal angiogenesis. *Bone* 2012; **51**: 535–45. doi: https://doi.org/10.1016/j.bone.2012.05.008
40. Farshad M, Würgler-Hauri CC, Kohler T, Gerber C, Rothenfluh DA. Effect of age on fatty infiltration of supraspinatus muscle after experimental tendon release in rats. *BMC Res Notes* 2011; **4**: 530. doi: https://doi.org/10.1186/1756-0500-4-530
41. Wong MD, Dorr AE, Walls JR, Lerch JP, Henkelman RM. A novel 3D mouse embryo atlas based on micro-CT. *Development* 2012; **139**: 3248–56. doi: https://doi.org/10.1242/dev.082016
42. Apps JR, Hutchinson JC, Arthurs OJ, Virasami A, Joshi A, Zeller-Plumhoff B, et al. Imaging invasion: micro-CT imaging of adamantinomatous craniopharyngioma highlights cell type specific spatial relationships of tissue invasion. *Acta Neuropathol Commun* 2016; **4**: 57. doi: https://doi.org/10.1186/s40478-016-0321-8
43. Hutchinson JC, Arthurs OJ, Ashworth MT, Ramsey AT, Mifsud W, Lombardi CM, et al. Clinical utility of postmortem microcomputed tomography of the fetal heart: diagnostic imaging *vs* macroscopic dissection. *Ultrasound Obstet Gynecol* 2016; **47**: 58–64. doi: https://doi.org/10.1002/uog.15764
44. Hutchinson JC, Ashworth MT, Sebire NJ, Arthurs OJ. Multiple cardiac rhabdomyomas visualised using micro-CT in a case of tuberous sclerosis. *Fetal Diagn Ther* 2017; **41**: 157–60.
45. Hutchinson JC, Ashworth M, Ramsey AT, Mifsud W, Lombardi CM, Sebire NJ, et al. Diagnostic accuracy of micro-CT for fetal heart dissection (comparison of imaging to autopsy). *Pediatr Radiol* 2016; **46**: S141.
46. Verleden SE, Vasilescu DM, Willems S, Ruttens D, Vos R, Vandermeulen E, et al. The site and nature of airway obstruction after lung transplantation. *Am J Respir Crit Care Med* 2014; **189**: 292–300. doi: https://doi.org/10.1164/rccm.201310-1894oc
47. Boon M, Verleden SE, Bosch B, Lammertyn EJ, McDonough JE, Mai C, et al. Morphometric analysis of explant lungs in cystic fibrosis. *Am J Respir Crit Care Med* 2016; **193**: 516–26. doi: https://doi.org/10.1164/rccm.201507-1281oc
48. Hutchinson JC, Barrett H, Ramsey AT, Haig IG, Guy A, Sebire NJ, et al. Virtual pathological examination of the human fetal kidney using micro-CT. *Ultrasound Obstet Gynecol* 2016; **48**: 663–5.
49. Siebert JR, Smith KJ, Cox LL, Glass IA, Cox TC. Microtomographic analysis of lower urinary tract obstruction. *Pediatr Dev Pathol* 2013; **16**: 405–14. doi: https://doi.org/10.2350/13-08-1359-oa.1
50. Fukuda M, Matsunaga S, Odaka K, Oomine Y, Kasahara M, Yamamoto M, et al. Three-dimensional analysis of incisive canals in human dentulous and edentulous maxillary bones. *Int J Implant Dent* 2015; **1**: 12.
51. Vasconcelos KF, Dos Santos Corpas L, da Silveira BM, Laperre K, Padovan LE, Jacobs R, et al. MicroCT assessment of bone microarchitecture in implant sites reconstructed with autogenous and xenogenous grafts: a pilot study. *Clin Oral Implants Res* 2016; **28**: 308–13.
52. Elfarnawany M, Alam SR, Rohani SA, Zhu N, Agrawal SK, Ladak HM. Micro-CT *versus* synchrotron radiation phase contrast imaging of human cochlea. *J Microsc* 2016; **265**: 349–57. doi: https://doi.org/10.1111/jmi.12507
53. Pereira RC, Bischoff DS, Yamaguchi D, Salusky IB, Wesseling-Perry K. Micro-CT in the assessment of pediatric renal osteodystrophy by bone histomorphometry. *Clin J Am Soc Nephrol* 2016; **11**: 481–7. doi: https://doi.org/10.2215/cjn.04810515
54. Tang R, Buckley JM, Fernandez L, Coopey S, Aftreth O, Michaelson J, et al. Micro-computed tomography (micro-CT): a novel approach for intraoperative breast cancer specimen imaging. *Breast Cancer Res Treat* 2013; **139**: 311–16. doi: https://doi.org/10.1007/s10549-013-2554-6
55. Tang R, Coopey SB, Buckley JM, Aftreth OP, Fernandez LJ, Brachtel EF, et al. A pilot study evaluating shaved cavity margins with micro-computed tomography: a novel method for predicting lumpectomy margin status intraoperatively. *Breast J* 2013; **19**: 485–9.
56. Tang R, Saksena M, Coopey SB, Fernandez L, Buckley JM, Lei L, et al. Intraoperative micro-computed tomography (micro-CT): a novel method for determination of primary tumour dimensions in breast cancer specimens. *Br J Radiol* 2016; **89**: 20150581.
57. Sarraj W, Tang R, Najjar A, Griffin M, Bui A, Zambeli-Ljepovic A, et al. Prediction of primary breast cancer size and T-stage using micro-computed tomography in lumpectomy specimens. *J Pathol Inform* 2015; **6**: 60.
58. Jawad N, Sebire NJ, Wade A, Taylor AM, Chitty LS, Arthurs OJ. Body weight lower limits of fetal postmortem MRI at 1.5 T. *Ultrasound Obstet Gynecol* 2016; **48**: 92–7. doi: https://doi.org/10.1002/uog.14948
59. Rutty GN, Brough A, Biggs MJ, Robinson C, Lawes SD, Hainsworth SV. The role of micro-computed tomography in forensic investigations. *Forensic Sci Int* 2013; **225**: 60–6. doi: https://doi.org/10.1016/j.forsciint.2012.10.030
60. Thali MJ, Taubenreuther U, Karolczak M, Braun M, Brueschweiler W, Kalender WA, et al. Forensic microradiology: micro-computed tomography (micro-CT) and analysis of patterned injuries inside of bone. *J Forensic Sci* 2003; **48**: 1336–42.

61. Richards CS, Simonsen TJ, Abel RL, Hall MJ, Schwyn DA, Wicklein M. Virtual forensic entomology: improving estimates of minimum post-mortem interval with 3D micro-computed tomography. *Forensic Sci Int* 2012; **220**: 251–64. doi: https://doi.org/10.1016/j.forsciint.2012.03.012
62. Cecchetto G, Amagliani A, Giraudo C, Fais P, Cavarzeran F, Montisci M, et al. MicroCT detection of gunshot residue in fresh and decomposed firearm wounds. *Int J Legal Med* 2012; **126**: 377–83. doi: https://doi.org/10.1007/s00414-011-0648-4
63. Cecchetto G, Giraudo C, Amagliani A, Viel G, Fais P, Cavarzeran F, et al. Estimation of the firing distance through micro-CT analysis of gunshot wounds. *Int J Legal Med* 2011; **125**: 245–51. doi: https://doi.org/10.1007/s00414-010-0533-6
64. Fais P, Giraudo C, Boscolo-Berto R, Amagliani A, Miotto D, Feltrin G, et al. Micro-CT features of intermediate gunshot wounds severely damaged by fire. *Int J Legal Med* 2013; **127**: 419–25. doi: https://doi.org/10.1007/s00414-012-0775-6
65. Fais P, Giraudo C, Viero A, Miotto D, Bortolotti F, Tagliaro F, et al. Micro computed tomography features of laryngeal fractures in a case of fatal manual strangulation. *Leg Med (Tokyo)* 2016; **18**: 85–9. doi: https://doi.org/10.1016/j.legalmed.2016.01.001